International Journal of Biochemistry Research & Review
3(2): 119-128, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Toxic Effects of Sub-Chronic Administration of Chloroform Extract of *Artemisia maciverae* Linn on the Kidney of Swiss Albino Rats

Atawodi Sunday Ene-ojo[1], Ene Aloy Chinedu[2] and Fatihu Muhammad Yakasai[3]

[1]Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria.
[2]Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[3]Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ASEO and EAC respectively designed the study. Author ASEO managed the analyses of the study. Author EAC did all the laboratory work, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author FMY carried out the histopathological analysis of the animals' organs. All authors read and approved the final manuscript.

Research Article

Received 17th August 2012
Accepted 26th October 2012
Published 5th April 2013

ABSTRACT

The nephrotoxic potential of sub-chronic doses of chloroform extract of *Artemisia maciverae* Linn was studied in male Swiss albino rats. The groups were respectively administered chloroform extract of *Artemisia maciverae* at 0, 50, 100 and 200 mg/kg b.wt for 60 days and then monitored till day 90 before sacrifice. Sera samples were analyzed for urea and creatinine. The kidneys were subjected to histological examination after staining with hematoxyline-eosin. At the onset of treatment, the extract caused statically significant ($p<0.05$) elevation in serum urea and creatinine. The mean (+SD) levels of serum urea at the onset of treatment with 0, 50, 100 and 200 mg/kg of the extract were 29.6+ 1.10, 54.1+4.40, 81.6+8.50 and 132.1+6.10mg/dL respectively, while that of serum creatinine were 0.5+0.10, 0.8+0.10, 1.2+0.10 and 1.3+0.30mg/dL respectively. The

**Corresponding author: Email: chineduene@gmail.com;*

elevations in serum urea levels returned to normal after the onset of treatment, but that of creatinine persisted. Thirty days after withdrawal of treatment, the levels of serum urea in the 0, 50 and 100 mg/kg treatment groups were found to be 30.1+2.00, 32.1+ 2.00 and 33.3+1.30mg/dL respectively, while that of serum creatinine were found to be 0.6+0.10, 1.2+0.10 and 1.2+0.10mg/dL respectively. Microscopically, tubular epithelial necrosis was observed in the treated animals in the early stages, but the renal injuries disappeared at the later stage. These results suggest that chloroform extract of *Artemisia maciverae* may be nephrotoxic at high doses.

Keywords: Artemisia maciverae; nephrotoxicity; Swiss albino rats.

1. INTRODUCTION

Artemisia maciverae Linn, a small herbaceous plant belonging to the family Asteraceae is one of the widely used medicinal plants in the northern part of Nigeria. It is known locally by the Hausa people of Nigeria as tazargade. The Hausa people of northern Nigeria use this plant traditionally in treating malaria. Various pharmacological studies have been carried out on this plant products which showed that the chloroform extract possess anti-malarial activity [1].

In as much as many health problems have been solved using medicinal plants, a number of them are toxic when not properly prepared or dispensed [2]. As such a scientific approach needs to be applied towards the use of plant extracts in managing ailments, especially in the developing countries where the level of literacy is low and the status of health management is poor, and about 80% of the population patronize herbal drugs. The toxicity potentials of the extracts of some medicinal plants have been documented. Some of these plants are *Artemisia annua* L [3], *Artemisia japonica* L [4] and many others. The workers reported that the oral administration of ethanolic extract of *Artemisia annua* L. adversely affected post implantation development and pregnancy in the rat when dosed from 35 to 75mg/kg [3]. In their own study with the ethanolic extract of *Artemisia japonica* Zhang *et al.,* reported that the extract containing artemisinin possesses no genetic toxicity at all levels [4]. From literature, nothing is known of *Artemisia maciverae* toxicity, hence this study.

The kidney is the primary organ for clearance and excretion of xenobiotics including drugs and drug products from the body. Damage to the kidney could arise due to the administration of plant extracts, but there is paucity of scientific information because the incidence of toxicity in local settings are hardly reported or documented. A study of the toxicity of *Artemisia maciverae* is imperative because of its widespread use in northern Nigeria. Moreover, a study of the effect of the drug extract on the kidney is essential because of the cardinal role the organ plays in plasma clearance, some detoxification, homeostasis and excretion of xenobiotics. The estimation of the histological effect on the kidney tissues and the determination of some waste metabolic products excreted exclusively via the kidneys provide useful information about the health status of the kidneys. Such metabolites include urea and creatinine [5]. Systemic electrolyte and water balance are regulated via the kidney, thus the plasma electrolyte levels also provide vital information about the functional state of the kidneys [6].The present study aims at establishing the possible nephrotoxic effect of the chloroform extract of whole plants of *Artemisia maciverae* which has been found to possess significant anti-malarial activity *in vivo*.

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

The plant *Artemisia maciverae* was collected in Zaria, Kaduna State, Nigeria, and identified by a Taxonomist at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

The whole plants of *Artemisia maciverae* were dried under the shade for two weeks and ground into powdered form using laboratory mortar. Extraction of this powdered form of the plant was carried out by first defating it with petroleum ether for 8 h before extracting with chloroform for (4 h x 2) using Soxhlet apparatus. The extract containing chloroform was allowed to evaporate to dryness to prevent its toxic effect. The chloroform extract of *A. maciverae* was selected from all the other `extracts for detailed toxicity study. This is because the chloroform extract of this plant exhibited the highest antimalarial activity compared to the methanol and petroleum ether extracts of *A. maciverae* [1]. The extract was stored in the refrigerator at -4ºC until required.

2.2 Animals and Treatments

Ninety six (96) male adult Swiss albino rats of about 12 weeks of age were randomized into four groups, each containing 24 rats. The rats were allowed to adjust to the laboratory environment for one week before the commencement of study. Group 1 which served as the normal control was administered 0.3% Tween 80, while rats in groups 2, 3 and 4 were administered the chloroform extract of *Artemisia maciverae* (dissolved in 0.3% Tween 80, ie the vehicle) at the dose levels of 50 mg/kg, 100 mg/kg and 200 mg/kg body weight (b.wt) respectively for sixty days. Ninety days is the standard duration for sub-chronic toxicity studies (sixty days for drug administration and thirty days for monitoring the animals after drug administration). The plant extract was administered to the animals in the treatment groups through the intraperitoneal route and also the normal control group animals were administered 0.3% Tween 80 through the intraperitoneal route. The four days duration for treating malaria infected animals has no correlation with the duration of sub-chronic toxicity studies. The doses were selected based on the results of a previous study by Ene *et al* (2008). At the end of 60 days, administration of extract was withdrawn and the surviving animals were monitored for another 30 days before being euthanized.

Through out the experimental period, all animals were observed daily for clinical signs and symptoms of toxicity. Animals were euthanized at weeks 0, 1, 2, 4, 8, 10 and 12. At the end of the study, all surviving animals were euthanized. The method of decapitation was used to euthanize the animals. Blood samples collected were allowed to stand slanted in the laboratory, and the serum separated by centrifuging at 3,000rpm for 20 mins. The sera samples were stored at -20ºC until required for biochemical analysis. Kidneys of both the dead and euthanized animals were removed and stored in 10% formal saline for histopatholgical analysis.

2.3 Histopathological Studies

The kidneys were embebbed in paraffin wax, sectioned at 5µm and stained with haematoxylin and eosin [7]. Detailed microscopic examination was conducted on the kidneys from both control and treatment groups.

2.4 Biochemical Analysis

Whole blood samples were collected and allowed to clot. The sera samples collected were stored at -20ºC or analyzed immediately for creatinine and urea based on the principles outlined by Fabiny and Ertingshausen [8] and Searcy et al. [9]. respectively, using standard biochemical reagent kits (Biosystems S.A; Costa Brava 30, Barcelona (Spain), Iso 13485-TUV Rheinland-Reg: SX 60010383 0001).

2.5 Statistical Analysis

Results generated were analyzed using Analysis of Variance (ANOVA).

3. RESULTS

Administration of chloroform extract of whole plant of *Artemisia maciverae* produced signs of toxicity like loss of appetite, loss of agility, dizziness and convulsion in the treated groups. These signs of toxicity were found to increase in severity as the dose increases. Mortality was recorded in the 50 mg/kg treatment group in week one of treatment. There was also mortality in the 100 mg/kg treatment group in week one and four of treatment. All the animals in the 200 mg/kg body weight (b. wt) treatment group died within week one of treatment with convulsions as a major observable sign of toxicity. In the course of this study two animals out of 24 died in the 50 mg/kg b. wt treatment group within one week (5 days), and six animals out of 24 died in the 100 mg/kg b. wt treatment groups within three weeks (21 days). No casualty was recorded in the control group. Generally, there was a drop in water and food consumption in the animals as the dose of the extract administered to them increases.

Serum clinical chemistry showed some consistent changes. Over the twelve (12) weeks treatment period, there were statistically significant ($p<0.05$) elevation in the levels of creatinine and urea in the treated groups compared to the normal control (Figs. 1 and 2). There was a statistically significant difference ($P< 0.05$) observed when these biochemical parameters in the treated groups were compared with the normal control. The increase in these biochemical parameters was more prominent in weeks one and two of treatment. The levels of serum urea at the onset of treatment with 0, 50, 100 and 200 mg/kg of the extract were 29.6±1.10, 54.1±4.40, 81.6±8.50 and 132.1±6.10mg/dL respectively, while that of serum creatinine were 0.5±0.10, 0.8±0.10, 1.2±0.10 and 1.3±0.30mg/dL respectively. A statistically significant difference ($p<0.05$) was observed in the levels of this biochemical parameters in the treated animals compared to the untreated controls at the onset of treatment. These abnormal elevations in serum urea levels returned to normal after the onset of treatment while the abnormal elevations in serum creatinine levels persisted. On day 90(ie week 12), the levels of serum urea in the 0,50 and 100 mg/kg treatment groups were found to be 30.1±2.00, 32.1± 2.00 and 33.3±1.30mg/dL respectively, while that of serum creatinine were found to be 0.6±0.10, 1.2±0.10 and 1.2±0.10mg/dL respectively. The level of these biochemical parameters after withdrawal of treatment and day 90 is equally presented in Figs. 1 and 2. The urea level was found to return to normal while the elevation in creatinine persisted in the different treatment groups (Figs. 1 and 2).

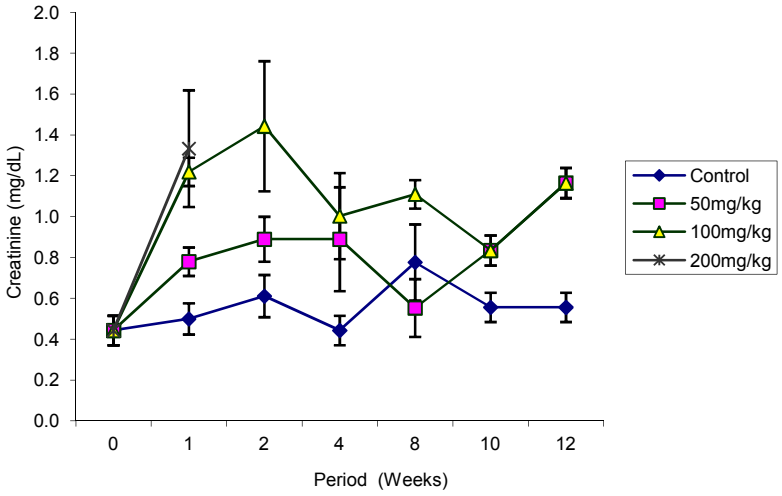


Fig. 1. Creatinine levels of rats treated with chloroform extracts of *Artemisia maciverae*

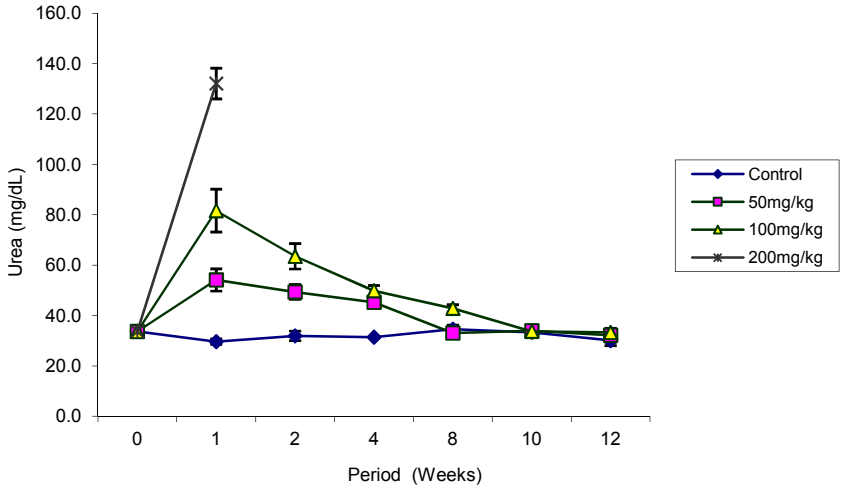
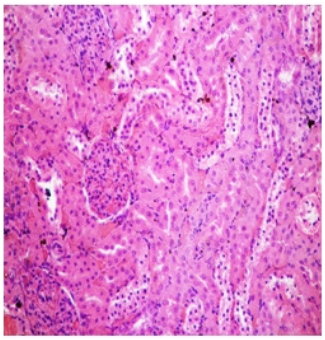


Fig. 2. Urea levels of rats treated with chloroform extracts of *Artemisia maciverae*

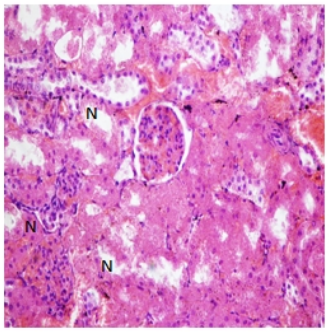
Gross and histopathological observations indicated congestion/tubular epithelial necrosis in the kidneys of the animals treated with 50 mg/kg, 100 mg/kg and 200 mg/kg of the extract (Plates 1), but not in the control, in week one of treatment. However, the lesions disappeared after 30 days of withdrawing treatment in the 50 mg/kg and 100 mg/kg treatment groups.

The weight of treated animals as compared to the control is presented on Table 1. Loss of weight in the treated animals especially in the 100 and 200 mg/kg treatment groups as compared to the control and 50 mg/kg group was observed. The percentage relative weight of the kidneys of the treated animals were significantly different ($p<0.05$) from that of the controls at the onset of treatment (Fig. 3). The significant increase ($p<0.05$) in percentage

relative weights of the kidneys of the treated animals at the onset of treatment when compared to the control is clearly demonstrated (Fig. 3).



Normal kidney of rat showing no lesion



Kidney of rat showing Tubular epithelial necrosis (N)

Plate 1. Histopathological appearances of the kidney of rats showing tubular epithelial necrosis (N) compared to normal kidney

Table 1. Average Changes in Body weight of rats receiving different intraperitoneal doses ofchloroform extract of *A. maciverae*

S/N	Dose (mg/kg)	N	Weight of rats (g)		Percentage weight gain (%)
			Initial	Final	
1	50	24	195.21±12.17	202.20±15.30	3.58
2	100	24	179.03±11.96	187.00±10.50	4.45
3	200	24	192.16±16.74	186.03±14.88	-3.19
4	Control	24	169.60±5.45	185.24±6.96	9.22

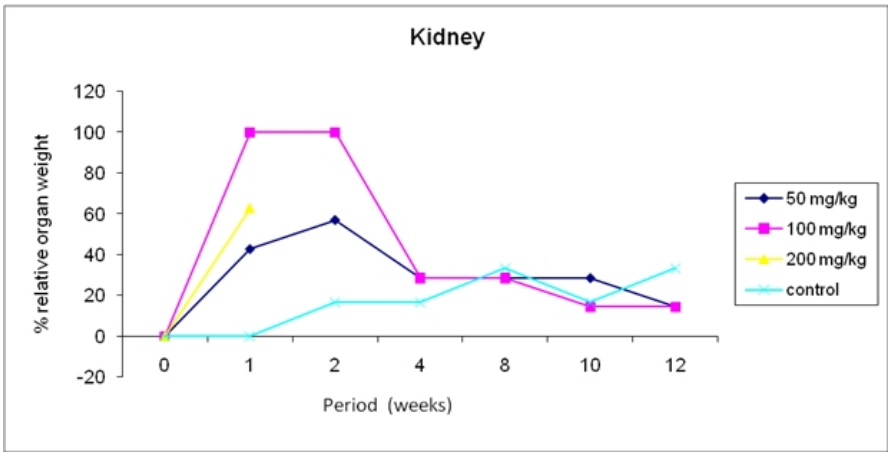


Fig. 3. Percentage relative weights of rats' kidneys treated with chloroform extract of *Artemisia maciverae*

The phytochemicals present in the chloroform extract of *A. maciverae* are flavonoids, triterpenes, terpenoids, glycosides, phlobatannins and tannins (Table 2).

Table 2. Phytochemical screening of the chloroform extract of Artemisia maciverae on aluminium oxide

General/Group tests	Specific tests	Result/inference
		A. maciverae (chloroform extract)
1) Test for flavonoids	a) Shinoda's test for flavones	-
	b) Sodium hydroxide test for flavonoids.	+
	c) Lead acetate test for flavonoids	+
	d) Ferric chloride test for hydroxyl group.	+
2) Test for cardiac glycosides	a) Lieberman Burkard test for triterpenes.	+
	b) Salkowskill test for terpenoids	+
	c) Keller-kiliani test for digitalis glycosides	+
3) Tests for anthraquinone derivatives	a) Borntrager's test for combined anthraquinone	-
	b) Borntrager's test for free anthaquinone	-
4) Test for phlobatannins	a) Hydrochloric acid test for phlobatannins	+
5) Test for tannins	a) Ferric chloride test for tannins	+
6) Test for saponins	a) Frothing test for saponins	-
7) Test for carbohydrates	a) Molisch's test for carbohydrate	+
	a) Barfoed's test for monosaccharide	-
	b) Fehling's test for reducing sugar	+
	c) Test for combined reducing sugar	+
8) Test for Alkaloids	a) Dragendroff's test	+
	b) Wagner's test	+
	c) Mayer's test	+

4. DISCUSSION

The therapeutic importance of the whole plant of *Artemisia maciverae* in folk medicine had been documented in our earlier studies [1]. Notwithstanding, there is paucity of information regarding the adverse or toxic effect of the plant extract in spite of its use in folk medicine practice. Current study showed that the chloroform extract of this plant could be potentially nephrotoxic when the dose is high and the duration of use extended.

This sub-chronic study revealed that the chloroform extract of *Artemisia maciverae* was toxic to the experimental animals during the experimental period particularly at week one (Figs. 1 and 2). This toxic effect was observed with all the doses of the extract administered to the animals. The extract was associated with consistent dose -and duration -dependent increase in serum urea and creatinine with significant increases ($p<0.05$) observed in the 50, 100 and 200 mg/kg treatment groups compared to the untreated controls. The toxic effect of this plant extract was reversed within 4 weeks of withdrawing treatment (Figs. 1 and 2) suggesting that the nephrotoxic effect of chloroform extract of *A. maciverae* may not be permanent.

With respect to serum biochemical parameters, the elevation observed at the onset of treatment in the levels of creatinine and urea may be attributed to the toxic effect of the plant extract on the kidney. The lesion inflicted on this organ by the plant extract might be responsible for the high values recorded in the above biochemical parameters during the first and second weeks of treatment (Figs. 1 and 2).

Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney. Therefore, marked increase in serum urea and creatinine are indications of functional damage to the kidneys [5]. At the onset of treatment, the elevation in the levels of urea and creatinine of the intoxicated rats, may indicate renal function impairment due to *A. maciverae* toxicity. This may be supported by the occurrence of renal lesions at the onset of treatment [10,11], as was the case in this experiment (Figs. 1 and 2; Plates 1). Although, urea level can be increased by many other factors such as dehydration, anti-diuretic, drugs, diet etc, creatinine is specific to the kidney since kidney damage is the only significant factor that increases serum creatinine level [12,13,14]. Therefore, the significant increase ($p<0.05$) in urea and creatinine levels at higher doses of the extract and when administration was conducted for a longer duration showed that the kidney was adversely affected by the extract, but the adverse effect was more prominent when high dose of the extract was given for a long period (Figs. 1 and 2).

Similar results were reported by AL-Sultan and Hussein [15]. In a toxicity study carried out with the ethanol extract of *Euphhorbia heliscopia* in Swiss albino rats, they reported that at the onset of the experiment, there was a significant increase in the levels of serum creatinine and urea. Furthermore, they observed lesions in the kidney of the animals in the treatment group during the study period. There was also a similar result reported by Ene et al. [16]. In a hepatoxicity study carried out with the chloroform extract of *A. maciverae*, in Swiss albino rats, the results indicate that long term exposure to therapeutic doses of chloroform extract of this plant is relatively safe, but high dose exposure may result in hepatocellular injury [16]. In another study by Ene et al. [17], this chloroform extract caused elevation in the PCV, hemoglobin, WBC and lymphocyte counts of the rats treated with 200 mg/kg of the extract at the onset of treatment. This suggests that there was some possible breach of the integrity of blood synthesis and regulatory system with high doses of the extract compared with the lower doses.

These abnormalities observed in the levels of serum creatinine and urea at the onset of the experiment was brought back to normal towards the end of the experiment when treatment was withdrawn. This may be explained by the fact that the liver was able to detoxify the extract at this stage. The detoxified compounds were handled through the excretion process of the kidney. In the histopatholgical evaluation of the tissues, lesions were observed in the kidney of the treated groups during the onset of the experiment. These lesions in the kidneys were found to disappear after the onset of the experiments even before treatments were withdrawn. The histopathological results at the onset and towards the end of the experiment concur with the results of the biochemical parameters. The elevation in the biochemical parameters duringthe onset of treatment (Figs. 1 and 2) concurs with the lesions observed in the kidney during the onset of treatment (Plate 1). The return to normalcy in the levels of the biochemical parameters towards the end of the experiment also concurs with the absence of lesions in the major organs of the animals at that period of the experiment, suggesting cessation of toxic effects of the plant extract.

The phytochemical studies of this extract shows that triterpenes and terpenoids are present (Table 2). These phytochemicals are also present in *Artemisia annua* L. Excess of these phytochemicals may result into toxic effect of the plant extract [3].

5. CONCLUSION

Evaluation of sub-chronic/nephrotoxic effects in rats dosed 50 mg/kg, 100 mg/kg and 200 mg/kg b.wt of *Artemisia maciverae* showed that the extract is toxic at the onset of treatment, but toxicity can be reversed by the withdrawal of treatment. It is therefore concluded that the chloroform extract of *Artemisia maciverae* may be nephrotoxic especially at higher doses and on chronic application.

ACKNOWLEDGMENT

Our gratitude goes to Mr. Apeh Y.E.O of the Biochemistry Department of Ahmadu Bello University, Zaria, Nigeria, for technical assistance, and Mr. Ugwu M.N, for typing the original manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ene AC, Ameh DA, Kwanashie HO, Agomo PU, Atawodi SE. Preliminary *in vivo* anti-malarial screening of petroleum ether, chloroform and methanol extracts of fifteen plants grown in Nigeria. J. Pharmacol. Toxicol. 2008;3(4):254-260.
2. Dalsiel JM. Botany, uses of *Alsonia boonei* stem bark In: The useful plants of West Africa. Crown agents for Overseas government and administration, London, 1997;260-264.
3. Boaretao AC, Muller JC, Bufalo AC, Botelho GG, de Araujo SL, Foglio MA, de Morais RN, Dalsenter PR. Toxicity of artemisinin (*Artemisia annua* L.) in different periods of pregnancy in Wistar rats. Reprod. Toxicol. 2008;25(2):239-46.
4. Zhang D, Cheng P, Ling L. Antioxidant and genetic toxicity of *Artemisia japonica* L. extract. Nat. Prod. Res. Dev. 2011;23(1):39-42.

5. Panda NC. Kidney In: Textbook of Biochemistry and Human Biology, 2nd ed. Prentise Hall, India. 1999;290-296.
6. Nwanjo HU, Nwokoro EA, Opara AU. Non-protein nitrogen In: Blood Chemistry, Theory, analysis and interpretation. 1st ed. Hacyn Publishers, Owerri, Nigeria. 2004;65-134.
7. Drury RAB, Wallington EA, Roy C. Carleton's histological technique, 4th Edition; Oxford University Press, London. 1993;49-98.
8. Fabiny DL, Ertingshausen G. Automated reaction-rate method for determination of serum creatinine with centrifichem. Clin. Chem. 1971;17:696-700.
9. Searcy RL, Reardon JE, Foreman JA. A new photometric method for serum urea nitrogen determination. Amer. J. Med. Technol. 1967;33:15-20.
10. Kelly WR. In: Veterinary and Clinical diagnosis, 2nd ed. W. R. Kelly (ed). Baillier Trindall, London. 1984;260-300.
11. Hassieb, MM, EL-Swak AA, Ali FR, Hussein YA. Phenylmercuric chloride toxicosis in rats. Alexandria J. Vet. Sci. 1988;4:509-521.
12. Cheesbrough M. Clinical chemistry tests, In: District laboratory practice in tropical countries, Cambridge new ed; part 1. 1998;331-336.
13. Chan PK, O' Hara GP, Hayes WA. Principles and methods for acute and subchronic toxicity. In: principles and methods of toxicology. Hayes W.A. (ed). Raven Press, N. Y. 1984;351-376.
14. Adeyemi OO, Elujoba AA, Odesanmi WO. Evaluation of the toxicity potential of Cassia podocarpa with reference to official senna. W. Africa. J. Pharmacol. and D. Res. 1988;8(1):41-47.
15. AL-Sultan SI, Hussein A Yehia. Acute toxicity of *Euphorbia heliscopia* in rats. Pakistan J. Nutr., 2006;5(2):135-140.
16. Ene AC, Atawodi SE, Ameh DA. Hepatotoxic effects of chloroform extract of *Artemisia maciverae* Linn in rats following intraperitoneal administration of different subchronic doses. Human Exp. Toxicol. 2011;30(1):25-33.
17. Ene AC, Atawodi SE, Ameh DA, Adamu S, Kwanashie HO. Changes in the hematological parameters of rats administered sub-chronic doses of chloroform extract of *Artemisia maciverae* Linn. Int. J. Res. Chem. Environ. 2012;2(3):106-112.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=213&id=3&aid=1212